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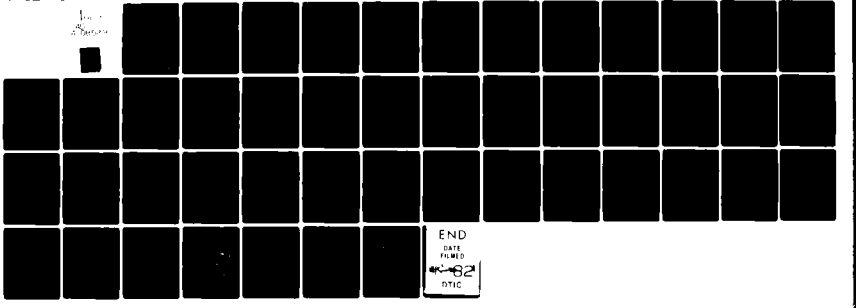

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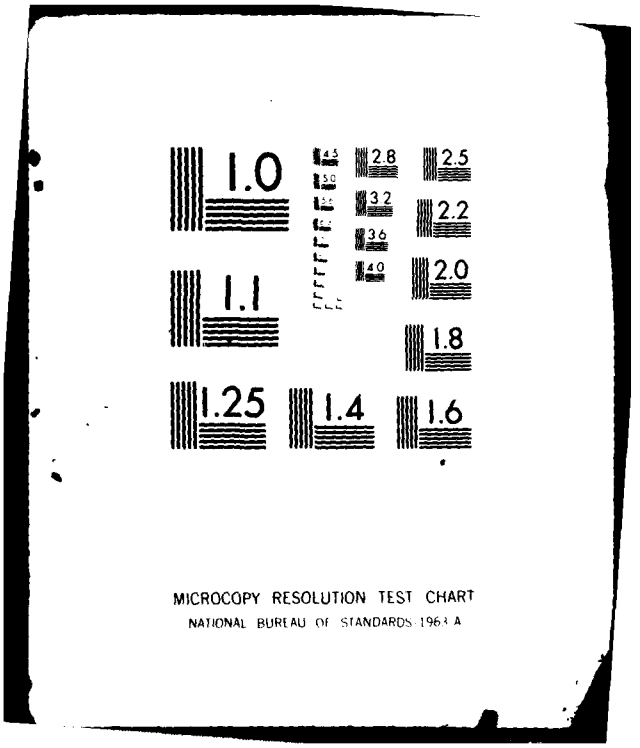

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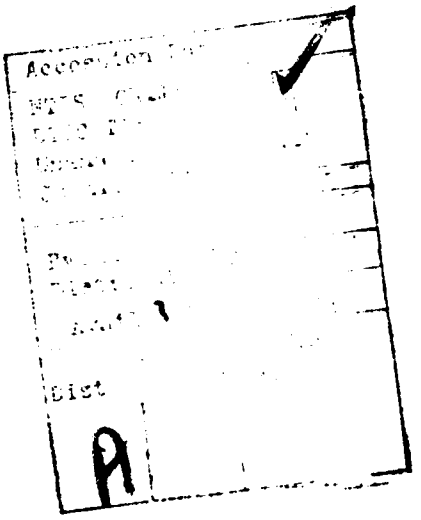

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THE USE OF ELEMENT-SPECIFIC DETECTORS COUPLED WITH HIGH-PERFORMANCE LIQUID CHROMATOGRAPHS

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ABSTRACT

Element-specific detectors (ESDs) provide an excellent means of determining the molecular character and elemental composition of complex column effluents, when used in tandem with HPLC systems. General purpose flame and electrothermal AA units along with inductively coupled and direct current plasma detectors are discussed in terms of the inherent advantages, difficulties, and detection limits associated with using such systems. Other, more limited ESDs, such as flame photometric detectors are also compared. The HPLC-ESD interface remains the largest hurdle to increased utilization of this new analytical probe and attempts to solve or minimize this problem are reviewed. Recent applications involving HPLC-ESD systems illustrate the important role that these detectors can and will play in solving a wide variety of speciation problems with biological, environmental, and energy-related matrices. Over 100 literature citations are provided.

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CONTENTS

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I. INTRODUCTION

Ever-increasing demands for data concerning the molecular structure or characterization of species which are present in a variety of complex matrices (e.g., fuels and by-products, biological media, saline solutions, soils, etc.) require two criteria to be met. First, separation techniques must be available that provide sufficient resolution to permit analytes of differing molecular structures to be resolved. For those cases where similar structures, especially geometric isomers, are involved, packing materials and chromatographic systems capable of very high resolution (N > 5000) are required. Advances in gas chromatography [1] and, more recently, high performance liquid chromatographs [2,3] meet this requirement. The second criterion involves the reliable and selective detection of the particular analyte(s) of interest often in minute quantities amid complex eluents.

As evidenced by this volume, many detectors are available which are capable of measuring a variety of atomic and molecular properties. Our intention in this chapter is to discuss the subset of detectors generally labeled as element-specific or element-selective detectors (ESD). Simply stated, within some predefined confidence interval, a detector capable of reproducibly detecting a unique property of an element or a particular molecular form of that element as neutral or ionic species will be considered to have element-specific or element-selective characteristics.

Detectors that measure atomic transitions are element-specific by the aforementioned definition. These include conventional flame and graphite furnace (electrothermal) atomic absorption [4,5], atomic emission [6], and atomic fluorescence [7] detectors. If one includes those detectors that form species which are distinguishable by molecular properties, more useful detection systems may be considered as element-specific. Thus, for example, flame photometric detectors [8] which chemically produce and detect HPO [9], S_2 [10], or SnH [11] as transient analytes are element-specific for phosphorus, sulfur, or tin, respectively [12].

Clearly, the definition of element-specificity could include a wider range of detectors and some have recently been applied to HPLC. Nuclear magnetic resonance detectors measure events at radio-frequencies which are characteristic for spin-active nuclei in various chemical environments [13,14]. Infrared detectors measure vibrational frequencies, many of which may be unique to specific functional groups and/or molecular configurations involving specific elements [15]. Valid arguments may be made for the selectivity of these and other detection systems relying on similar principles of detection. Typically, however, these systems are also very sensitive to the analyte's chemical environments, and measurements quite often must rely on both intra- and inter-molecular electronic interactions between the several atoms ill-defined interactions with the matrix. This is particularly true for detection in condensed phases, examplified by HPLC mobile phases. Since these measurement techniques are more suitably included in other subject matter in this book, our discussion in this chapter is limited to those detectors whose output can be correlated with the chemical behavior of specific elements present in HPLC effluents where such matrix or background effects are minimized.

An illustration of the analytical advantages of using ESDs is shown in Figure 1. The employment of non-specific mass detectors (e.g., refractive index (RI)) provides detectable signal for many both important and non-important analytes, regardless of their chemical content. The use of other non-specific detectors that measure some molecular property (e.g., UV or ultraviolet absorbance (UV) at selected wavelengths) are incapable of detecting materials that do not possess the needed electronic configuration. Thus, analytes containing the element of interest may remain undetected. ESD systems provide an excellent probe for detecting all eluting species that contain the selected element of interest. Since capability for simultaneous detection of many elements exists, valuable data on the elemental composition of each resolved species may also be collected.

The importance of ESDs in analytical determinations is quite evident. The authors are also aware that data pertaining to the chemical environments of target elements, obtained by non-specific detectors, may serve an important supplemental role. However, one must first reliably determine the presence of a particular target element in the resolved chromatographic peak. ESDs perform that function quite well under many difficult circumstances.

II. EXPERIMENTAL CONSIDERATIONS FOR COUPLED HPLC-ESD SYSTEMS

A. On-line Versus Off-line Detection

Efficient methods of transporting chromatographically resolved analytes into the operating environment of the detector are critically important in analytical procedures. Transportation of candidate analytes may be accomplished by employing a direct feed of all or a preselected portion of eluant solution from the liquid chromatographic system to the ESD. Conventional nonelement-specific UV and RI detectors [16], and element-specific ones such

as flame atomic absorption (FAA) [17,18], inductively coupled plasma [6], and direct current plasma [19] detectors provide good examples of demonstrated directly-coupled HPLC-detector supporting systems.

Where interfacing of the HPLC to the ESD has not been achieved, off-line or indirect characterization remains a viable alternative. The general approach to such indirect characterizations involves the use of fraction collectors which sequentially accumulate predetermined volumes of eluent from the HPLC effluent [20]. These fractions are then individually carried into the detector for determination by manual [21] or automated methods [22].

Iverson et al., [23] have speciated inorganic arsenic and organo-arsenic compounds by HPLC by incorporating off-line graphite furnace atomic absorption (GFAA) spectrometry for detection. Their results, which conveniently map arsenic concentration as a function of vial number, are basically similar to the much higher resolution histograms generated by Fish et al. [24] for arsenic species using a more sophisticated directly-coupled HPLC-GFAA system (Figure 2). However, direct coupling is a more desirable approach since arsenic concentration may be continually monitored from a dynamic, continuous flow system where virturally no opportunity for contamination is possible. Data so obtained, can provide accurate information on elemental concentrations in the eluent as a function of time. Moreover, chromatograms of species having close retention volumes (V_R) or elution times (t_R) may provide erroneous information on the nature of analyte species unless sample volumes of collected fractions are carefully regulated [21] and are very small with respect to flow rate, e.g., five percent.

Selective fraction collection, (i.e., the collected volumes are sufficiently discrete to contain only one analyte), however, may be used to advantage quantitatively. Vickrey et al., [21] developed a system where the LC

effluent is selectively trapped. After the portion of effluent which contains the analyte of interest was passed through a UV detector, a six-way valve was used to divert up to 2.4 mL of effluent into a storage tube. The effluent stream was then redirected to its normal flow pattern. Stored increments of the analyte were then transported via a peristaltic pump to an electrothermal AA detector for elemental analysis.

The collection of fractions containing the entire eluted species improves detection limits, provides more AA analyses for better profiling an eluting species, and eliminates restrictions in flow rates for analytes having concentrations contained in small volumes of analytes. Unfortunately, peak broadening due to the large volume (30.5 cm x 0.05 cm i.d.) of the storage tube employed, and the limitation that retention volume data must be approximately known beforehand, present problems that must be considered when using this method.

The method of choice, therefore, involves direct coupling of the ESD to the LC column whenever it is possible to do so. Ramifications of such interfaces are discussed in the next section.

B. Interfacing HPLC Systems to ESD's

The simplest interfaces between HPLC columns and ESD's involve direct coupling of the sample stream to the analytical chamber of the detector (Figure 3). Small bore teflon tubing, which satisfactorily minimizes peak broadening and zone spreading, has been directly connected to nebulizers, thereby requiring few modifications to the detector [25].

Efficient resolution of chromatographic events is dependent in part on flow rates used to elute analytes of interest [26]. Important information may be lost in a laminar system when detector responses are slower than the operational time scale required of a detector [16]. Moreover, detector sensitivity may be sacrificed when experimental conditions germane to resolution

require fast flow rates to be used. For their FAA detector, Botre et al., [27] removed the internal flow spoiler on their nebulizer to permit more effluent (ca. 80 percent) to be analyzed. Their solvent system composed of 60 percent methanol and 40 percent water did not hamper the efficient operation of the flame. Van Loon et al., [28] coupled an HPLC to a non-dispersive atomic fluorescence detector (NDAF); the modified nebulizer and burner system could easily accommodate flow rates up to 4 mL min^{-1} of aqueous or dilute salt solutions. However, the limited use of other solvents such as hydrocarbons [29] may be dictated by their compatibility with the detector under consideration.

Interfacing an HPLC to a GFAA detector presents a challenge to the analyst. Since samples injected into the GFAA must undergo preparative drying and charing steps prior to atomization and detection, the sample stream cannot enter the detector under continuous flow conditions. Brinckman et al., [22] circumvented this problem by designing a unique flow-through sampling cup. With the aid of a commercial automatic sampler (Figure 4), the post-column HPLC solvent stream continually flows through a teflon cup with a 50 µL capacity. Depending on operating parameters selected for the GFAA detector (dry, char, atomization, and furnace cool-down times), 20 µL aliquots of column effluent may be periodically quantitated at intervals (Δt) as frequent as Δt = 45 s.

In the case of an ideal chromatograph-ESD system, where the column effluent is continuously monitored, the resolved analyte produces a Gaussian curve indicating concentration as a function of time [22]. For the HPLC-GFAA system, a series of histograms indicates analyte concentration at specific time intervals. The sum of these sets of individual events may be related to the total amount of each analyte injected into the HPLC-ESD system. Two quantative methods of measurement have been analyzed. One method relies on connecting the tops of the individual AA peaks and, by planimetry, measuring

the enclosed area. The alternative method involves simply summing the AA integrands for an eluting peak. Figure 5 indicates that results from using both methods are directly comparable ($r = 0.999$). The method of choice, thus, is the latter since it requires less time for data reduction. Moreover, results from either determination are directly proportional to the amount of analyte injected into the HPLC-ESD system. Vickrey et al., [21,30,31] have devised a pulsed sampling GFAA apparatus which operates from external flags on their HPLC microprocessor controller. Analytical results obtained from the Vickrey method are similar to those obtained by Brinckman et al., [22,32] and Parks et al., [33].

Hill and Crist [34] have demonstrated a novel nitrogen detector whose HPLC interface consisted of the column effluent being carried on a moving wire to an oven. There the sample was oxidized, subsequently carried to a reaction furnace, and quantitatively detected using a Hall-type conductivity meter. The same interface was also used to couple HPLC to a thermal ionization detector [8].

Thus, many techniques are now available which facilitate the tandem coupling of HPLC to ESD systems. Selection of the most appropriate interface ultimately depends on the individual operational parameters and sampling requirements of the chosen ESD.

III. ELEMENT-SPECIFIC DETECTORS--INHERENT ADVANTAGES AND DISADVANTAGES

A. Commonly Employed Specific Detectors

The bulk of literature concerning element-specific detectors has been directed to electrothermal and flame single-element detectors, and direct current and inductively coupled plasma multi-element detectors. Here, these four categories of detectors are compared with respect to the analytical advantages and problems of direct coupling to HPLC systems. Each detector has

a number of advantages which may be exploited by analysts to solve specific analytical problems. Conversely, difficulties such as matrix effects and compatibility of the detector with various flow rates and solvent systems, arising with coupled systems, are critical for successful HPLC applications and must be considered in some detail.

Analyses by conventional AA spectrometers require conversion of the analyte to the atomic form usually by thermal means in a flame or furnace. The flame is supported by a combination of gases selected to optimize detection of a specific element. Depending on the analysis, traits of either technique may dictate its preferred use. Where concentrations of analytes are in the $\mu g\ mL^{-1}$ range or greater, FAA spectrometry is normally the method of choice. Primary consideration lies in the simple interface required for HPLC-FAA that permits direct analysis of the laminar effluent stream [15]. Continuous monitoring results in the generation of expected Gaussian curves [16,35,36] for the resolved analytes.

Table I clearly indicates that GFAA or Zeeman atomic absorption (ZAA) detection limits are two to three orders of magnitude better than those of FAA. Moreover, only 5 to 10 percent of the sample is destructively evaluated by the pulsed mode of operation characteristic of HPLC-GFAA systems [21,22,30,33]. The remainder of the analyte may be analyzed on- or off-line by other isotope-selective detectors, such as mass spectrometers [37-39] and nuclear magnetic resonance spectrometers [12-14]. However, if the analyte is contained in an eluting volume less than one sampling interval, Δt, that particular analyte may elute undetected by the ESD analytical probe [21,22].

Both FAA and GFAA are generally restricted to single element analyses, although independent reports by Felkel and Pardue [40] and Harnly et al. [41] demonstrate the use of both FAA and GFAA multi-element spectrophotometers.

Table 1

Detection Limits of Selected Elements by Various Element Specific Detectors ($\mu g\ mL^{-1}$)

Element	FAA (a)	GFAA (a)	ZAA (b)	DCP (c)	ICP (d)
As	0.1	0.00006	0.0005	0.030	0.030
Cr	0.003	0.000005	0.00045	0.001	0.006
Cu	0.002	0.000008	0.0005	0.001	0.002
Pb	0.010	0.0003	0.0002	0.015	--
Se	0.1	0.0001	0.05	0.030	0.015
Zn	0.0006	0.0000007	0.000005	0.005	0.005

(a) All FAA and GFAA data taken from reference [42].
(b) All ZAA data taken from reference [43].
(c) Except for Zn (reference [6]) all DCP data taken from reference [19].
(d) Except for Zn (reference [42]) all ICP data taken from reference [6].

Gross differences in atomization procedures and absorbance wavelengths for electrothermal analyzers and changes in burner gas composition for flame systems that are required for the various elements, cause delays in analyses of more than one element. This problem becomes more formidable when considering multi-elemental analyses of an HPLC effluent. Sequential multi-elemental analyses using single element detectors may be approached by two methods: (1) using the previously discussed collection of fractional volumes of HPLC effluent [21], and (2) using repetitive sample injections with the appropriate optimized conditions for each element. However, increased analysis times and departure from more automated approaches present serious liabilities for these techniques.

During the last two decades, significant effort has been directed to the development of several rapid sequential and simultaneous multi-element detectors. Microwave induced plasma (MIP) [44], direct current plasma (DCP) [45], and inductively coupled plasma (ICP) detectors [46-49] have been developed. Of these detectors, only MIP detectors have not been successfully interfaced with liquid chromatographic systems. This is primarily due to the inability of the MIP detectors to operate stably with the bulk liquid flow rates normally used in HPLC [44]. Microwave-induced plasma detectors are typically stabilized at relatively low gas pressures (< 10 torr); hence, rapid volatilization of HPLC carrier solvents would swamp the detector. However, a recent report of the ongoing development of a MIP equipped with a Beenakker cavity [50,51] and LC membrane interface which can operate at atmospheric pressures suggests future promise for the incorporation of this detector into a HPLC-ESD system [52].

DCP and ICP detectors represent multi-element detectors which are capable of <u>in situ</u> monitoring of several elements contained within a single analysis [53] (Figure 6). Prospects for direct determination of analytes' empirical formulae thus appear good. Although solvents commonly used for ion exchange or

reverse phase separations may be carried directly into the plasma via the standard ceramic nebulizer chamber, problems may occur with similar introduction of hydrocarbon and halocarbon solvents [29,55].

Uden et al., [29] designed an aerosol nebulizer interface to circumvent these difficulties and have been successful in utilizing, for example, hexane with various polar modifiers such as methylene chloride, diethyl ether or acetonitrile to resolve complexed metal species by an HPLC coupled to a direct current plasma.

B. Other Element-Specific Detectors

Although the majority of literature citations for ESD's fall under the categories of AA, ICP, and DCP spectroscopy, there exist other detectors which are considered to be element specific. Generally, these detectors have either found limited use as ESDs or are very restricted in the variety of elements that are detectable. Nonetheless, these detectors may be very useful for specialized applications.

Nitrogen, phosphorus, and sulfur represent examples of elements that may be specifically analyzed with detectors of limited versatility. The previously mentioned [34] nitrogen-selective detector for HPLC was used as an analytical tool for the rapid screening of many nitrogen-containing pesticide residues. Column flow rates had to be adjusted in order to arrive at the proper compromise between analysis time, degree of separation desired, and signal sensitivity. Relationships between peak area and pesticide injected were found to be linear and limits of detection approached 0.2 ppm, depending on the chemical composition of the analyte.

Julin et al. [56] developed a flame emission detector selective for phosphorus (526 nm) and sulfur (383 nm) emissions. This detector uses a special burner assembly to handle the total liquid effluent from HPLC columns

(e.g., 0.5 to 2.0 mL/min) and relies on generation of excited sulfur and phosphorus species in a hydrogen-rich flame [11]. Achieved detection limits for sulfur of 20 ppb and phosphorus of 2 ppb are reported. Similar detection limits are reported for these species by McGuffin and Novotny [57]. Moreover, further optimization of this system may lead to a further increase of an order of magnitude in sensitivity.

Concurrent with the work of Julin et al. [56], Freed has reported on the general use of flame emission spectroscopy as a detector for alkaline and lanthanide series metal species in tandem with on-line HPLC separations [58]. Detection limits (e.g., Na = 0.1 ppm) are quite good by this technique.

Compton and Purdy [8] modified a flame ionization detector to an act as a thermal ionic detector. With this detector they achieved enhanced selectivity of phosphorus over hydrocarbons and improved sensitivity. Capability for examining nitrogen- and sulfur-containing compounds by this technique is also possible.

Other more general detectors include flame resonance [59], non-dispersive atomic fluorescence (NDAF) [7], molecular cavity emission analysis (MECA) [60], and ion-selective electrodes [61]. NDAF detectors provides simultaneous multi-element detection with detection limits of one to three orders of magnitude greater than those achieved by FAA [62] and interfacing the fluorescence detector to HPLC systems is a simple task. However, matrix interferences may severely limit the application of NDAF detectors unless chromatographic schemes are devised which effectively separate candidate analytes from NaCl and other such interferring matrix components.

MECA remains a potential detector for HPLC, but no such system has been reported. This may arise because direct laminar introduction into the MECA cavity cannot be easily accomplished. The analyte is injected into a cavity

at the end of a stainless steel rod and the cavity is then rotated into a nitrogen diluted hydrogen flame for analysis. This sample injection technique is remarkably similar to the HPLC-GFAA sampling method [22]; therefore, the design of an interface working on a principle similar to that discussed by Brinckman et al. [22] should be possible in this case. The biggest drawback to on-line MECA appears to be that analysis times needed for different elements [60,63] to gain improved sensitivity are variable. A compromise between sensitivity and flow rates must therefore be made.

One may argue for the inclusion of ion-selective electrode detectors [61] in the general class of ESDs based on the proven selective measurement of, for example, Cl^- in the presence of a variety of potential interfering ions (i.e., PO_4^{3-}, SO_4^{2-}, CO_3^{2-}, NO_3^-, ClO_4^-, and OAc^-). However, only in rarest of cases would one opt to use this detector over the many other more efficient and sensitive ESDs described herein. Thus, capability now exists for direct coupling of both single- and multi-element selective detectors to HPLC systems under specific conditions of solvent and flow rate. After qualitative considerations have been met, one must consider how much analyte is needed for a particular analysis. This will be considered in the next section.

IV DETECTION LIMITS OF HPLC-ESD SYSTEMS

The absolute minimum concentration that an analyte can be detected is a significant feature of all analytical determinations. Conscientious researchers who examine, for example, various forms of metal species in complex matrices report that a particular species is either detected or not detected. The difference between "not detected," "not present," is very important, for as great strides in lowering detection limits occur, heretofore undetected concentrations of analyte in candidate matrices are brought within the reach of the improved analytical instrumentation.

Simply stated, every detector has a finite lower limit of detection, based on the particular analyte to be determined and--in some cases--the matrix which holds that analyte. Every manufacturer publishes his own list of sensitivities and, with appropriate modifications in optics and electronics, claims specific limits of detection. For detectors with the same properties these numbers generally fall fairly close to one another. So the attempt here is to illustrate the general differences for the various types of detectors as they pertain to six elements--three of which (As, Pb, and Se) appear in various inorganic and organometal forms as pesticides [20,23,64], herbicides [33], and commercial wastes. The other three elements (Cu, Zn, and Cr) are considered trace essential elements which are necessary for the proper functioning of the human biological system [65-67]. Results are summarized in Table I.

Several observations may be made from these data if one keeps in mind the facts that (1) numbers indicated therein represent the use of solutions containing very little, if any, matrix interfering materials, and (2) numbers, although chosen from a particular instrument manufacturer, are generally well within an order of magnitude of those values reported by manufacturers of similar instrumentation. Only two methods are used to render the analyte into an atomic or ionic state. One method involves the resistance heating of a graphite sample cell or cuvette up to 3000 °C. GFAA and ZAA are representative of this method of atomization. The major difference between the two approaches lies in how the respective optical systems process the signal [68,69]. Thus, one would expect similar detection limits to be obtained and this is found to be the case. Most elements are within a half an order of magnitude of each other, as determined by the two approaches. (Table I).

The other approaches to atom or ion generation include FAA, DCP, and ICP. Depending on the element to be analyzed, FAA incorporates a mixture of gases

that are used to generate a flame of desired characteristics. Both DCP and ICP rely on production of an "electrical" flame which results in temperatures of 5000 to 6000 °C in the hottest useful chemical reaction flame (nitrous-oxide/acetylene) [42,48,70]. Detection limits for elements which are atomized at low temperatures are of the same magnitude in both flame and plasma techniques (see Table I) while difficult to atomize elements (B,P) are significantly more sensitive using plasma techniques.

One must approach a discussion of detection limits, not as numbers generated by a specific detector when simple or uncomplicated matrices are involved, but more realistically as a total "system" detection limit. Normally, HPLC-ESD system detection limits will be higher than those for the detector alone. For example, if a 2 ppm solution of an element in a particular form can be ascertained at a 2σ confidence level for a given number of determinations, it may take 10 to 20 times as much analyte, all other things being equal, to arrive at a comparable figure of merit when the detector samples the effluent from an HPLC. This decrease in detection limit when incorporating the HPLC-ESD system is mainly due to (1) dilution of the analyte as part of the chromatographic process, and (2) influences in sensitivity that the "new matrix" e.g., the HPLC solvent system, may have on the detector.

Perhaps too much discussion has been devoted to detection limits. "Dirty" samples or analytes contained within complicated "real-world" matrices almost always have higher detection limits than analogous analytes in simple matrices. ZAA in some cases, provides the only exception to this rule since matrix effects and loss in sensitivity due to the employment of background correction have been significantly minimized [43]. Moreover, avenues are available which provide chromatographers with methods of handling very dilute quantities of analytes. May et al. [71] developed preconcentration techniques for polynuclear aromatic hydrocarbons on C_{18} reverse bonded-phase columns. Using a similar

column packing, Blair et al. have demonstrated a technique whereby alkyltin cations may also be preconcentrated from aqueous media prior to detection [72]. Ion exchange chromatography was used by Sandhu and Nelson to concentrate and separate arsenic from polluted water [73]. There is a definite need for additional preconcentration technology to be advanced for other elements of interest to environmental, industrial, and other research areas.

Cassidy et al. preconcentrated organosilicon compounds from industrial process waters [74]. Packing materials of Tenax-GC (60 to 80 mesh), Porapak Q or Porapak N (80 to 100 mesh) were dry packed into 7 mm i.d. glass columns, and connected to a sample reservoir. The collected organosilicons were then eluted with methyl-iso-butyl ketone.

Biechler successfully preconcentrated trace Cu(II), Pb(II), Zn(II), Cd(II), Ni(II), and Fe(III) salts on a Chelex 100 (50 to 100 mesh) resin [75]. Prior to preconcentration, metal salts were buffered to a pH ~ 5 with ammonium acetate buffer. Elution of the concentrated metal species was then effected by using 4N nitric acid.

Vickrey et al. [31] have developed a technique which uses post-column sample collection. Here the volume of solvent containing the analyte is stored in discrete (100 µL or 220 µL) intervals and later 37 µL of each analyzed by ZAA. This method provides a more frequent sampling interval whereby 17 to 37 percent of the total analyte is carried into the electrothermal furnace instead of the 5 to 10 percent reported earlier by Brinckman et al. [22].

The preceding series of examples involving preconcentration and post-column collection are used to put the concept of detection limits in proper perspective. Of overriding importance in choosing any analytical technique should be the capability of the chosen HPLC-ESD system to analyze a particular element contained in a complex matrix under the HPLC column conditions required to

separate one compound from another. The rapidly-increasing methodology for preconcentration of samples of post-column sample manipulations thus renders consideration of detection limits less important.

V. APPLICATIONS OF HPLC-ESD SYSTEMS

Literature contributions that involve the utilization of general purpose mass selective or non-specific detectors for HPLC are prominent. Well-established procedures that are capable of examining a great variety of environmentally, industrially, and biologically significant organic materials that are contained in simple matrices have been reported. In contrast, applications of HPLC-ESD systems involving "real" samples, (i.e., analytes contained in complex environmental media or biological fluids) are substantially fewer in number. These examples that realize the potential of such powerful new analytical methodology are elegantly carried out and much of that work will be cited herein. Moreover, precursory demonstrations of the potential of HPLC-ESD systems in discerning the role of metal- and metalloid-containing species in their naturally occurring environments will also be addressed.

Selected applications of HPLC-ESD systems listed on an element by element basis are compiled in Table II. Contained therein are examples of the important role played by various HPLC-ESD systems in analyzing some 19 elements contained in a variety of chemical environments. More detailed examples listed under the separation technique (i.e., ion exchange, size exclusion, reverse bonded phase) employed are discussed below.

A. Ion Exchange Chromatography

Use of ion exchange chromatography with element-specific detectors has long been a method of choice for separation of many main group, transition, and lanthanide metal ions. This separation method was enhanced by forced flow liquid chromatographic systems utilizing fairly large diameter packing materials

Table II
Selected Applications of HPLC-ESD Systems

Element	ESD	Sample Matrices	LC Solvent Systems	LC Columns	References
As	ICP GFAA ZAA	99. and organic solutions of inorganic and organo-arsenic compounds, soils, plant tissues, oil shale retort waters	30:70 MeOH/H_2O with 1% hexadecylammonium bromides heptanesulfonic acid with HOAc in MeOH/H_2O, $(NH_4)_2CO_3$ in MeOH/H_2O, C_7H_{16}	ion pairing, RP-C_{18} AX,CX	22-24, 32, 63, 64, 78-80
Au	FAA	aq. solns. of Au anionic complexes	75% aq. NH_4OH	AX	81
Cd	FAA	inorg. CD^{2+}, Cd chelates, Cd in waste waters	NH_4OAc soln.	SEC, i.e., exchange	73, 82, 83
Cl	Ion selective electrode	Cl in sedimentary rocks		AX	61
Co	FAA,ZAA,DCP	Co complexes, inorg. CO^{2+} as the nitrate cyanocobalamin	EtOAc, $CHCl_3$, 2MNH_4OAc, 2-PrOH 2-BuOH, hydrocarbons, CH_3Br/C_2Cl_4 1:1	AX, adsorption, C_{18}	29, 43, 45, 55, 73, 84
Cr	FAA, DCP	Cr III & Cr VI complexes, bisarene chromium iodide, natural waters	C_6H_6:Me_2CO:H_2O, 2:7:1, 2-PrOH, 2BuOH, hydrocarbons	partition, AX, C_{18}	29, 55, 83, 85-87
Cu	FAA, NDIR AF, ICP, DCP	Cu-complexes waste waters	2-PrOH, 2-BuOH, MeCl, EtOAc, hydrocarbons	WAX, SCX, C_{18}, adsorption	24, 19, 35, 36, 45, 55, 83, 88
Fe	FAA, ICP	inorg. & organic Fe II & Fe III compounds, waste waters	H_2O, H_2O/EtOH, ϕCH_3, NH_4OAc (aq)	RP-C_8, ion exchange, adsorption	55, 73, 79
Hg	GFAA, ICP DCP	inorg. Hg^{2+} and RHgX (R = alkyl, x = halide) compounds, Hg complexes	2-mercaptoethanol in MeOH/H_2O, hydrocarbons 0.05 M NaBr/EtOH (2:1)	RP-C_8, C_{18}, adsorption	22, 29, 79
Mo	ICP	organomolybdenum carbonyl complexes	EtOH/H_2O	RP-C_8	79
N	nitrogen selective detector	nitrogen containing pesticides	Me_2CO, C_6H_{12}/Me_2CO	adsorption	34
Ni	FAA, ICP ZAA, NDAF	Ni complexes, waste waters	0.05 M $(NH_4)_2$ SO_4 aq., IVH_4OAc, NH_4NO_3(aq.)	SAX, IE, RP-48 adsorption	28, 29, 45, 55, 73, 89
P	FPD, Flame Emission	MeOH solutions or organo-phosphates, inorg. phosphates	HCO_2H	SAX, adsorption	56, 57
Pb	ZAA, ICP FAA	R_4Pb in synthetic mixtures & commercial gasolines, waste waters, chelates	EtOH/H_2O, MeOH/H_2O, C_6H_{14}, CH_2Cl_2, CH_3OH CH_3CN/H_2O	RP-C_{18}, adsorption AX	21, 22, 31, 73, 79, 83, 90-92
Pt	FAA	aq. solutions	aq. NH_4OH	AX	81
Se	ZAA	organoselenium compounds	MeOH/H_2O	C_{18}	30
Si	FAA	organosilicon monomers and polymers	MIBK/MeOH, THF/CH_3CN	SEC, RP-C_{18}	33, 74
Sn	GFAA, ZAA	R_4Sn, R_3Sn^+, R_2Sn^{2+} (R = alkyl), Sn polymers	MeOH/H_2O, EtOH, THF/CH_3CN	RP C_{18}, RP C_2	93, 94
Zn	FAA	waste waters, Zn-humic acid complexes	NH_4OAc (aq) TRIS Buffer	SEC, IE	73, 89

Legend:
AX = anion exchange
CX = cation exchange
IE = ion exchange
RP = reverse bonded phase
SCX = strong cation exchange, etc.
WAX = weak anion exchange, etc.

[76]. Recent advances in the production of stable microparticulate ion exchange stationary bonded phases [77] have now facilitated analytical procedures for separating and detecting nanogram and, in some cases, picogram quantities of inorganic and organometallic ions. The importance of element-specific detectors in providing rapid trace level characterization of column effluents cannot be overemphasized. This feature will become evident in the following illustrations.

Better elution behavior of metal ions typically may be achieved when ligands are exploited which impart selectively reduced ionic charge or more organic character to those analytes. Thereby ionic strengths (μ) of mobile phase can be reduced since the column retardation factor k' is proportional to $1/\mu$, with concommitant reduction of column degradation by excessive salts. These organic ligands, which form chelates or other similar complexes, may be added to analytes prior to injection into the HPLC-ESD system or may be part of the solvent system used to elute the analyte. The latter approach requires rapid equilibria to be established between analyte and mobile phase, but in most cases this presents little difficulty. Clearly, consideration must be given to the effect of this added matrix on the detection of candidate species.

Fraley et al. treated copper(II) ions with basic solutions of ethylenedinitrilotetraacetate (EDTA) and nitrilotriacetate (NTA) prior to injection into an Aminex A-14 anion exchange columns. By using a solvent system of 0.05 M $(NH_4)_2SO_4$, complete separation of $Cu(NTA)^-$ and $Cu(EDTA)^{2-}$ was attained [88]. Comparison of detector sensitivity for the complexed copper by inductively coupled plasma with conventional ultraviolet absorption at 254 nm indicated that ICP gave slightly more than an order of magnitude better sensitivity than the non-specific UV detector.

In a somewhat novel approach to determining the amount of various chelating agents in solution, Jones and Manahan reacted the indicator metal,

copper(II) with a solution containing EDTA, NTA, 1,2-cyclohexylenedinitrilo-tetraacetic acid (CDTA), and ethylene-bis(oxyethylenenitrilo)tetraacetic acid (EGTA) [36]. Each chelate gave a characteristic retention index (k') when eluted with a 0.05 M $(NH_4)_2SO_4$ solution which was passed through a weak anion exchange resin. Detection limits which were determined by flame atomic absorption spectrophotometry for $Cu_2(EGTA)$, $Cu(NTA)^-$, $Cu(EDTA)^{2-}$, and $Cu(CDTA)^{2-}$ were found to be 0.09, 0.108, 0.196, and 3.0 mg L^{-1}, respectively. Here, the relatively high value for $Cu(CDTA)^{2-}$ was attributed to its large k'. This finding is consistent with the fact that the longer an analyte is retained on a column substrate, the magnitude of zone spreading increases [35]. The concentration of analyte in the effluent becomes smaller, thereby requiring the injection of high quantities of analyte for detection.

Van Loon et al. reported on the separation of Cu(II), Ni(II), and Zn(II) complexes of EDTA, glycine, and trien [28] on a strong cation exchange micropartculate column [77]. Metal complexes contained in the effluent were detected by non-dispersive atomic fluorescence spectroscopy. Advantages of this detector lie in its multi-element capability (three hollow cathode lamps are used simultaneously) and the lower detection limits as compared to those obtained with conventional FAA spectroscopy. These authors, however, fail to indicate the magnitude of the difference in detection limits between NDAF and FAA detectors.

Slavin and Schmidt explored the use of metal labeling to improve the detection limit for amino acids [25]. Copper ions were complexed with histidine and eluted from a strong cation exchange column with NH_4NO_3. FAA spectroscopy was found to provide a detection limit of about 50 nm and the prospect of directly coupling the more sensitive GFAA detector should allow picogram quantities of complexed histidine to be determined. These detection limits are superior to those that are accomplished with UV detectors.

The molecular form of the analyte may have a significant effect on HPLC-ESD system detection limits. This effect is, in part, due to the chromatographic method of separation. For example, ion exchange usually involves the separation of charged elemental or molecular species of low volatility [77]. Thus, in the case of GFAA detection, the risk of partial volatilization due to drying, and charring procedures is minimized. Conversely, reverse bonded-phase separations of ionic analytes entail conversion to species of more covalent or organic character as ion pairs [96], which increases volatility and thus increases the likelihood of some loss through mobilization prior to atomization. This idea is borne out in studies involving the speciation of inorganic arsenic and organoarsenic compounds for which electrothermal GFAA and ZAA detectors are used. System detection limits using silica-based or resin based anion exchange columns with ammonium citrate or ammonium carbonate buffers were found to be as low as 5 ng for some species [24,32,80]. Comparable elution of these arsenic species by ion-pairing agents on a reverse phase column was at least an order of magnitude less sensitive [32].

The ability of ion exchange substrates to indefinitely retain metal species while preconcentrating those species from very dilute solutions has been demonstrated in a number of applications. Kahn and Van Loon [81] used a strongly basic anion exchange resin for on-column concentration of 100 ng amounts of platinum and gold ions from dilute liter solutions containing those ions. Since the volume of solution containing the analytes was not an important factor in the amount of analyte retained on the column, the prospect of using even more dilute solutions is a viable and important consideration in using ion exchange as a preconcentration analytical tool. The retained analytes were then eluted with 75 percent aqueous ammonium hydroxide and complete chromatographic resolution of the platinum and gold species was attained.

Using FAA as a detector directly interfaced with the anion exchange column, the authors found that 98 percent of the analytes contained in dilute solutions could be quantatively eluted [81].

Beichler demonstrated on-column concentration of Cu(II), Pb(II), Cd(II), Zn(II), and Ni(II) ions with an anion exchange resin [75]. One liter solutions of a mixture of the various ions produced nearly quantitative yields when comparing the quantity of metal ion added to solution to the amount of metal ion as determined by flame AAS. Similar approaches have been used for on-column concentration of arsenic [73] and chromium [87].

B. Size Exclusion Chromatography

Many notable advancements have been made in ESD applications involving high pressure or gravity flow size exclusion columns for separation. Materials that may be separated by this technique range from hydrated metal ions to large molecular weight metallo-polymers. Sufficient discretion in column performance is available whereby hydrated metal ions varying in radii by as little as 0.09 Å may be resolved [97]. An excellent review of SEC as it pertains to the separation of small and moderately sized inorganic compound has been prepared by Yoza [98]. Most of the molecules reported therein contain one or more elements amenable to analysis by ESD systems. SEC-ESD therefore offers a unique and exciting new tool for investigations of biologically important macromolecules such as metalloproteins, or environmentally important compounds in fossil fuels such as metalloporphyins, where key or diagnostic elements are integrally bound at low concentrations in the organic framework.

Size exclusion chromatography has been used in tandem with several AA detectors utilizing both on-line and off-line methods. One such system is reported by Yoza and Ohashi [99] where SEC is coupled to an FAA spectrometer. The authors designed a simple valving network to balance the column flow rate

to the flow rate carried into the nebulizer. This procedure avoided detector problems normally associated with gradient flow.

Properties of SEC gels have led to their general use in several areas. In 1962, a brief report by Hummel and Dreyer appeared [100] on the binding of 2'-cytidylic acid to pancreatic RNAase on a Sephadex G-25 column. With an eluent of 0.1 M acetate (pH = 5.3) solution, these authors were able to demonstrate that a 1:1 complex was formed by the two compounds. Although their detecton by UV spectrophotometry does not reflect our general topic element specificity, the principles espoused in this early work have stimulated further use of SEC for related analyses which involve characterization of metal-containing complexes and macromolecules [4].

One such application involves the speciation of analytes that are not readily detected by ESD systems. Kouchiyama et al. used magnesium as an elemental tag in their chromatographic determinations of polyphate complexes [101] on a Sephadex G-25 column. According to the properties exhibited by SEC, the elution order of analytes is such that larger molecules elute faster than smaller ones, barring any maverick adsorption of analytes onto the column substrate [98]. Polyphosphates obey this rule with the order of elution dependent on the molecular size of the phosphate moiety. An examination by FAA of collected fractions of eluent indicated that in each case magnesium(II) ions formed a 1:1 complex with the polyphate species. The total concentration uncomplexed magnesium ions were dependent on the molar amount of polyphates injected into the system. Micromolar quantities of analytes were readily characterized by this method.

One important characteristic of this SEC procedure lies in the fact that on-column derivitization may be accomplished [102]. This method both facilitates elution of the analyte and also provides a speciation tag for the

molecular characterization of materials whose elemental composition makes detection by ESD's either difficult or impossible.

Size exclusion chromatography has been substantially employed in examinations of the coordination of metal ions to ligands of biological and/or environmental significance. Segar and Cantillo [26] examined the coordination between ionic copper(II) and sea water samples. Data so obtained leads to estimation of the complexing capacity of sea water toward copper(II) in terms of the molecular weight range of dissolved compounds present in solution. Associated metal containing species were readily determined by atomic absorption spectroscopy. Clearly, this approach may be extended to include other metal ions of equal or greater biological and environmental impact.

The coordination of copper(II), zinc(II), and nickel(II) to humic acids and related ligands using SEC was studied by Mantoura and Riley [89]. Stability contraints for the various metal-containing species were estimated by measuring the concentrations of both free metal ions and complexed metal. Macromolecules present in the column effluent were detected by an on-line UV-visible spectrophotometer. A fraction collector was used to sequentially collect 1.3 mL aliquots of sample which in turn were examined by an off-line atomic absorption spectrophotometer.

Application of SEC to the study of metal binding to biological substrates (e.g., proteins or enzymes) is illustrated in the work of Voordouw and Roche [103] who examined the binding of calcium ions to the enzyme, apothermolysin. Their approach consisted of a modification of the technique reported by Hummel and Dreyer [100], which incorporated an FAA spectrometer as an ESD for calcium. When the pH of solution was varied, they found that the coordination of calcium(II) to apothermolysin involved simultaneous coordination or disproportionation of two calcium ions. Data obtained in this manner are important in determining the function or performance of biologically significant materials.

Element-specific detection is finding increased use in the characterization of metal-containing macromolecules [89,101-104]. For cases where the service of a material may be dependent on the molecular weight distribution of metal-containing moieties, SEC-ESD provides convenient separation and element-specific detection of such species. Parks et al. [33] characterized a number of marine antifouling formulations which contained several organotin moieties that were covalently bound to various polymeric substrates. The molecular weight range of tin-containing fractions were examined by tandem on-line UV, RI, and GFAA detectors. This approach leads to the determination of more effective polymer formulations.

C. Normal and Reverse Phase Columns as Used with ESDs

Proper selection of complexing or chelating agents coupled with metal ions has been shown to effect separation of metal species in normal and reverse phase columns. In many cases, the ligands employed may totally reduce the charge on the metal to zero, thus forming a neutral complex.

Stockton and Irgolic [96] were successful in speciating several inorganic and organoarsenic species employing sodium heptanesulfonate or sodium dodecylbenzenesulfonate used with microparticulate C_{18} reverse phase column. An electrothermal ZAA detector was interfaced to a 10 μm C_{18} column operated in the reverse phase mode. In this case the sulfonates actually serve as ion pairing agents to facilitate elution of the arsenic species of interest. Concentrations of 1 μg of each analyte were used in this study. Although the authors give no indication of the detection limits in this paper, their data and data presented in a subsequent paper indicate that the detection limit for arsenic species is on the order of about 100 ng. In comparison, improved detection limits over those attainable with reverse phase ion pair chromatography have been obtained for both silica based (> 10 ng injected as

As) [32] and resin based (5 ng injected as As) [80] columns. For each of the latter two cases, GFAA was employed as the element specific detector.

Jones and Manahan employing a high performance absorption column directly coupled to a FAA spectrometer speciated a series of neutral chromium complexes [85]. Standard solutions containing 800 ng each chromium acetylacetonate [$Cr(AcAc_3)_3$], chromium hexafluoroacetonate [$Cr(HFAA)_3$], and tris (2'hydroxyacetophenone) chromium [$Cr(HAP)_3$] were eluted within 5 min of injection. The incomplete resolution of $Cr(HAP)_3$ and $Cr(HFAA)_3$ suggests that either better elution parameters (e.g., change of flow rate, solvent system, and/or analytical column) should be selected or perhaps a change to a more sensitive ESD such as GFAA or ZAA may be used to optimize k's by permitting the use of smaller quantities of analytes, and thereby improving resolution by increasing the ratio of active sites on the column to the total amount of sample injected onto the column.

Uden et al. developed speciation of Co(II), Ni(II), Cu(II), Hg(II), and Cr(III) by using preinjection derivation of these species followed by HPLC-DCP analysis [29]. Metal ions were reacted with aqueous sodium diethyldithiocarbonate solution. Neutral complexes so formed were then separated by either reverse phase or adsorption chromatography. Solvents used for absorption chromatography separations were found to extinguish the plasma, requiring the generation of an aerosol without the aid of argon flowing at fast rates. Thus, the authors exhibit an aerosol-nebulizer interface capable of overcoming some of the difficulties encountered when selected solvent matrices are converted to aerosols and subsequently atomized in plasmas or flames.

VI. FUTURE PROSPECTS FOR HPLC-ESD SYSTEMS

In any endeavor, success is based on a series of predevelopmental steps that are critical to attaining the current state of achievement. The development

of viable HPLC-ESD systems is no exception to this generality. Such systems are currently in their early stages of development and many problems still prevail.

Complications involving matrix interferences that originate from the inherent disposition of target species and from solvent systems employed to effect desired separations must be minimized. Factors such as solvent gradients, salts, ligands, and dissolved particles may severely limit the use of ESD systems. Experiments concerned with the introduction of column effluents into various ESD systems via different nebulizer/spray chamber techniques provide valuable information on the efficiency of particle transport into these detectors [105].

Analytes dissolved in dilute, sub ppm or ppb, concentrations require detection systems that are suitable for sensitive, rapid on-line detection, and quantitation. This problem is being attacked in a number of ways. Reports are appearing on the development of preconcentration [73,74,81,87,106-109] and post-column derivatization [78,110,111] techniques which significantly improve detection limits. For GFAA detectors, batch off-line treatment of graphite tubes with refractory transition metal salts significantly improves detection limits for Sn, Se, and As [112], and a recently reported HPLC-GFAA interface now automates this system [113]. Improvements in detector sensitivity are also being made. For example, the use of coherent radiation sources such as tunable lasers [114] in place of discharge lamps, significantly lower detection limits. Bolshov et al. [115] have recently reported an excellent detection limit for lead [0.05 parts per trillion] by laser atomic fluorescence spectrometry.

Demonstration of a unique capability of element-selective detection was performed by Weiss et al. [93] and Jewett and Brinckman [94]. These researchers

measured the chromatographic capacity factor, k', for neutral R_4Sn species (reverse phase), charged R_3Sn^+ and R_2Sn^{2+} species (cation exchange) and ionic inorganic and organoarsenic(V) species (anion exchange) using HPLC-GFAA. The significant outcome from these experiments is reliable prediction k' for substances not detectable by conventional non-ESD means, based on independently derived molecular substituent parameters.

Depending on the separation process (i.e., ion exchange versus reverse phase) two molecular substituent parameters were employed. For reverse phase separations involving R_4Sn species appropriate values of the hydrophobic structural substituent (π) were linearly correlated to the natural logarithm of observed k' values. This parameter is based on a comparison between parent and derivative compounds with respect to their partitioning properties between octanol and water [116]. Weiss et al. found that linear regression of $\ell nk'$ versus π for R_4Sn species gave excellent correlations ($r = 0.989$) [93].

Ion exchange separations of charged arsenic and tin species by anion and cation exchange, respectively required the use of σ^{ϕ}, a parameter derived from the aqueous ionization of many substituted phosphorous acids [117]. Again good linear correlations between observed $\ell nk'$s and σ^{ϕ} values for inorganic- and organoarsenic compounds ($r = 0.971$) [93], and R_2Sn^{2+} and R_3Sn^+ compounds ($r = 0.969$ and 0.992, respectively) were found [94]. Such determinations suggest far-reaching implications on the predictive capabilities of HPLC-ESD systems for the determination of unknown organometals contained in a variety of complex matrices, for example, the speciation of arsenic in oil shale retort waters as demonstrated in the present case [93].

The greatest barrier, and perhaps the most difficult to overcome, to effective HPLC-ESD systems remains the development of efficient interfaces. Many nebulizer systems rely on partial transfer of LC effluents into detectors [105].

Other interfaces are ill-suited to remove those materials (solvents, salts, etc.) that interfere with the performance of a specific detector. Recent advances in the development of total consumption detection systems and other improvements in nebulizers [92,118] coupled with improved atomization efficiences offer attractive prospects for improved detection limits [56].

Literature citations generally provide evidence that the aforementioned analytical roadblocks are rapidly being minimized or overcome. Even under current experimentally imposed limitations, important data in environmental, energy, and biologically related areas occupy a prominent place in chemical literature. With the advent of future improvements, the role of ESDs should become even more important.

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Figure Captions

1. Three hypothetical chromatograms of a solution containing seven components are compared. The conventional HPLC-UV analysis (at top) reveals that five separated components bear chromophores. Tandem or separate element-specific detectors for elements X or Y, run either sequentially or simultaneously as a multi-ESD (X + Y), show simpler, more informative results: (a) elements X and Y are contained in peaks F and G, respectively, which are molecules bearing no chromophoric moieties; and (b) peak B (shaded) is a molecule containing both elements X and Y in a measurable ratio and also bears an active chromophore.

2. Comparison of two techniques for sampling an LC effluent stream. A. 500 ng of each arsenic species were injected onto a cation exchange column, collected in 4 mL fractions, and determined off-line by FAA [23]. B. Periodic stream sampling of several arsenic compounds (10 ng each) separated by anion exchange and automatically detected on-line by GFAA [22]. (A) reproduced by permission of the American Chemical Society.

3. Typical FAA interface where the LC column effluent is transported from an LC column through an aerosol-generating commercial nebulizer and into the flame for atomization [25]. The efficiency of transport is dependent on several factors including solvent and flow rate. Reproduced by permission of J. Chromatogr. Sci.

4. A representation of the carousel graphite furnace (GF) sample holder of the AS-1 auto-sampler is depicted. In this pulsed (periodic stream sampling) mode, the AS-1 sampling pipette traverses the arc (---) between the GF tube orfice (at arrowhead) and the conical aperture to a flow-through well sampler. Thus 10 to 50 µL effluent samples are reproducibly and periodically introduced for automatic, programmed GFAA analysis at the chosen wavelength. Reproduced by permission of J. Chromatogr. Sci. [22].

5. Comparison of two methods for quantifying speciated inorgnaic and organometal compounds. One method (abscissa) involves measuring the enclosed areas in the series of histograms that define and eluting sample peak. The alternative method (ordinate) involves the summing of the individual AA histograms that define an eluting sample.

6. Experimental demonstration of element-specific detection of macromolecules chromatograhically resolved by HPLC [54]. Whereas the UV detector only indicates the rpesence of species with chromophores, simultaneous multi-element detectors provide information concerning the elemental composition of these resolved species. Reproduced by permission of the American Chemical Society.

FIGURE 1

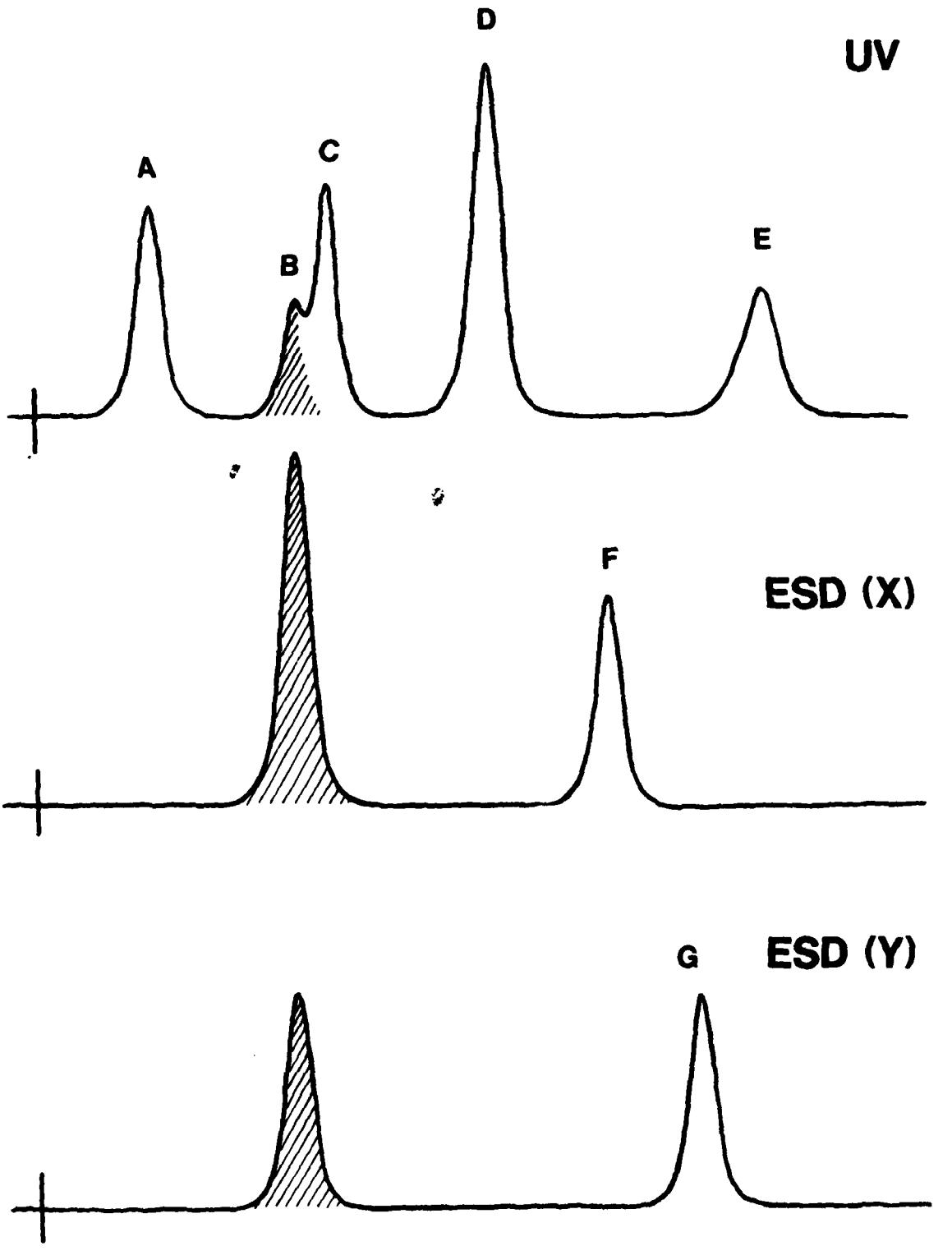
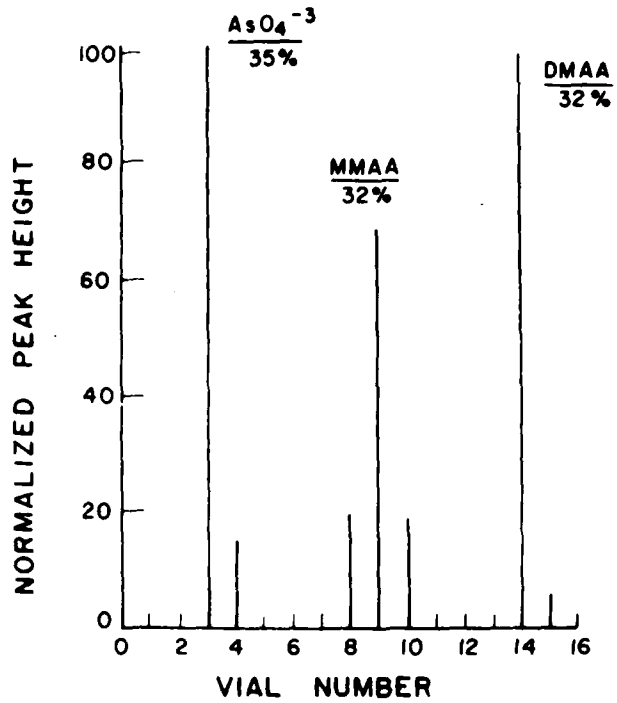


FIGURE 2

(A)


AsO_4^{-3}
35%
MMAA
32%
DMAA
32%
NORMALIZED PEAK HEIGHT
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16
VIAL NUMBER

(B)

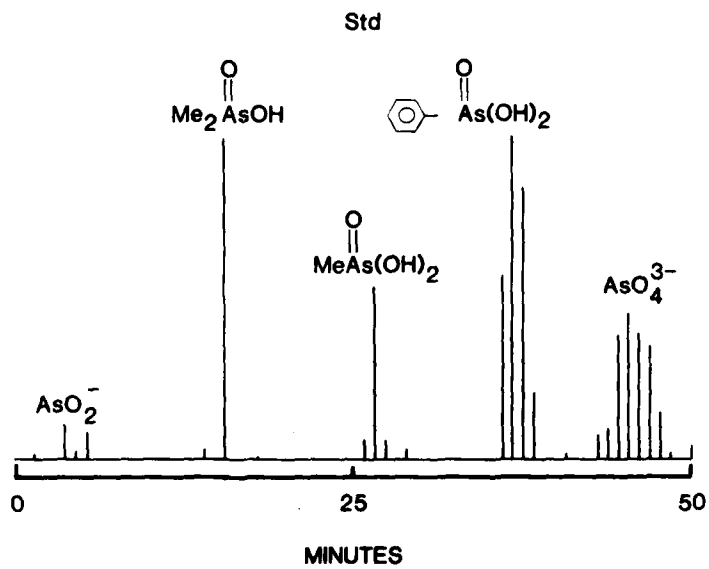

Std
Me_2AsOH
$As(OH)_2$
$MeAs(OH)_2$
AsO_4^{3-}
AsO_2^-
0
25
50
MINUTES

FIGURE 3

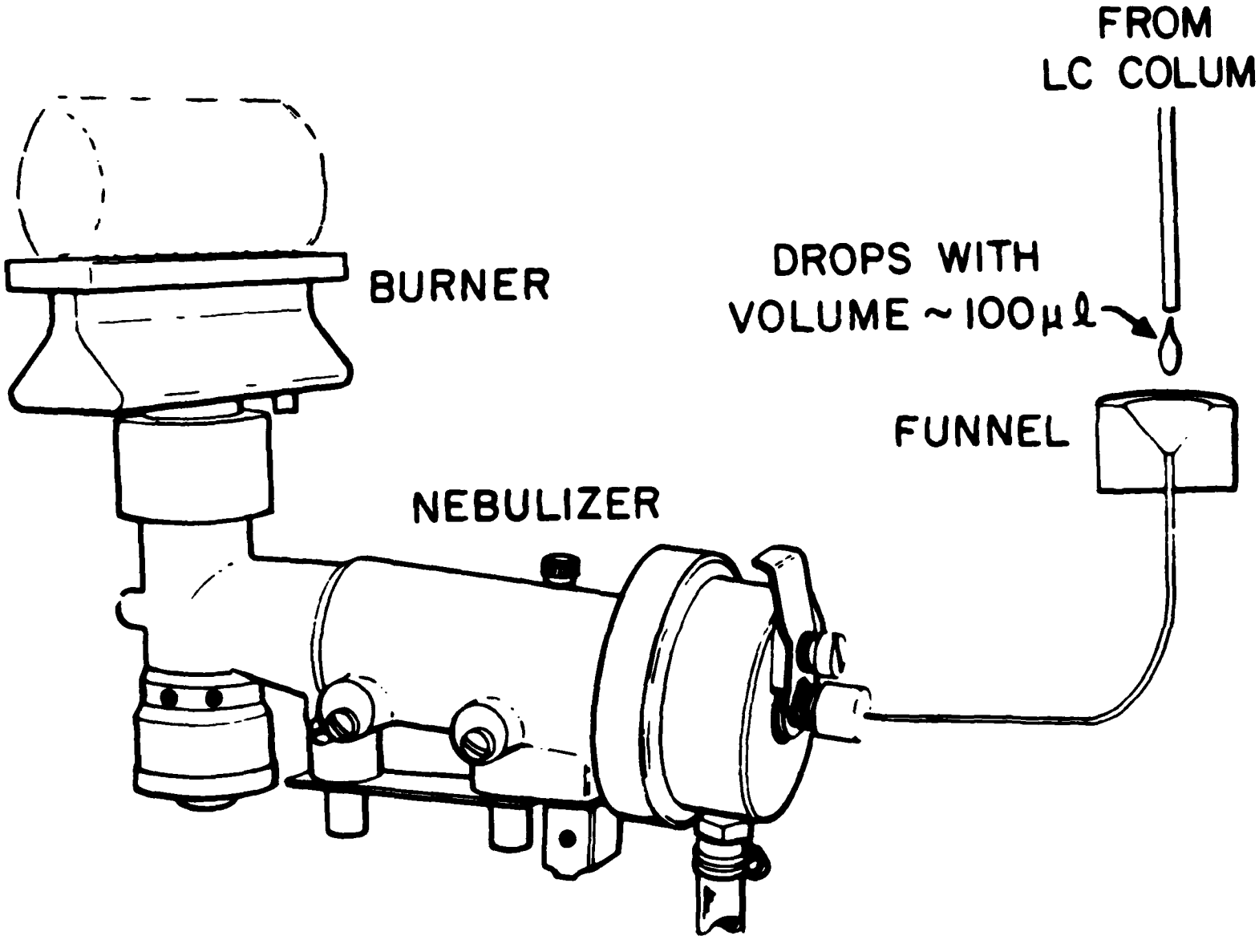

FROM
LC COLUMN
BURNER
DROPS WITH
VOLUME ~ 100µl
FUNNEL
NEBULIZER

FIGURE 4

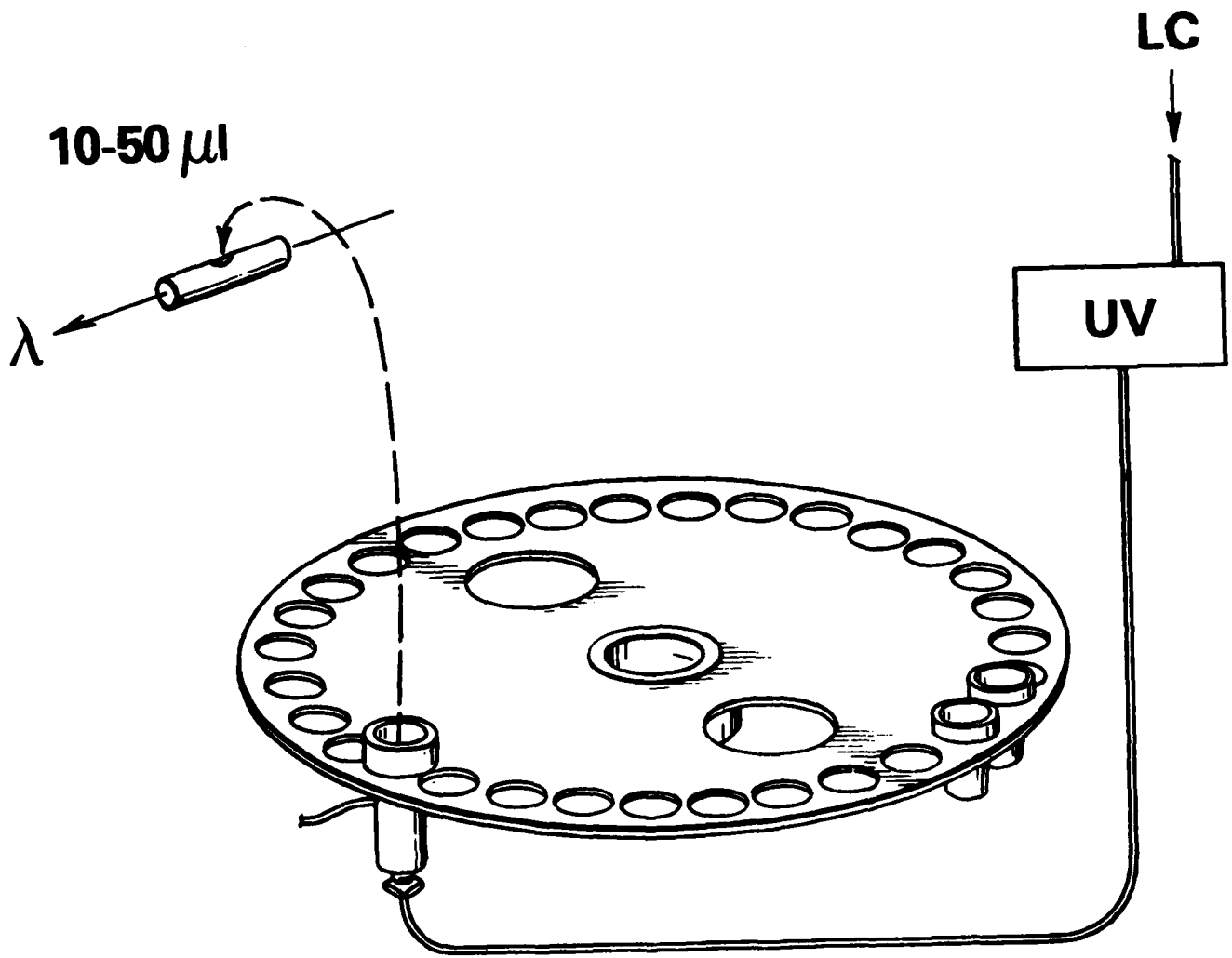


FIGURE 5

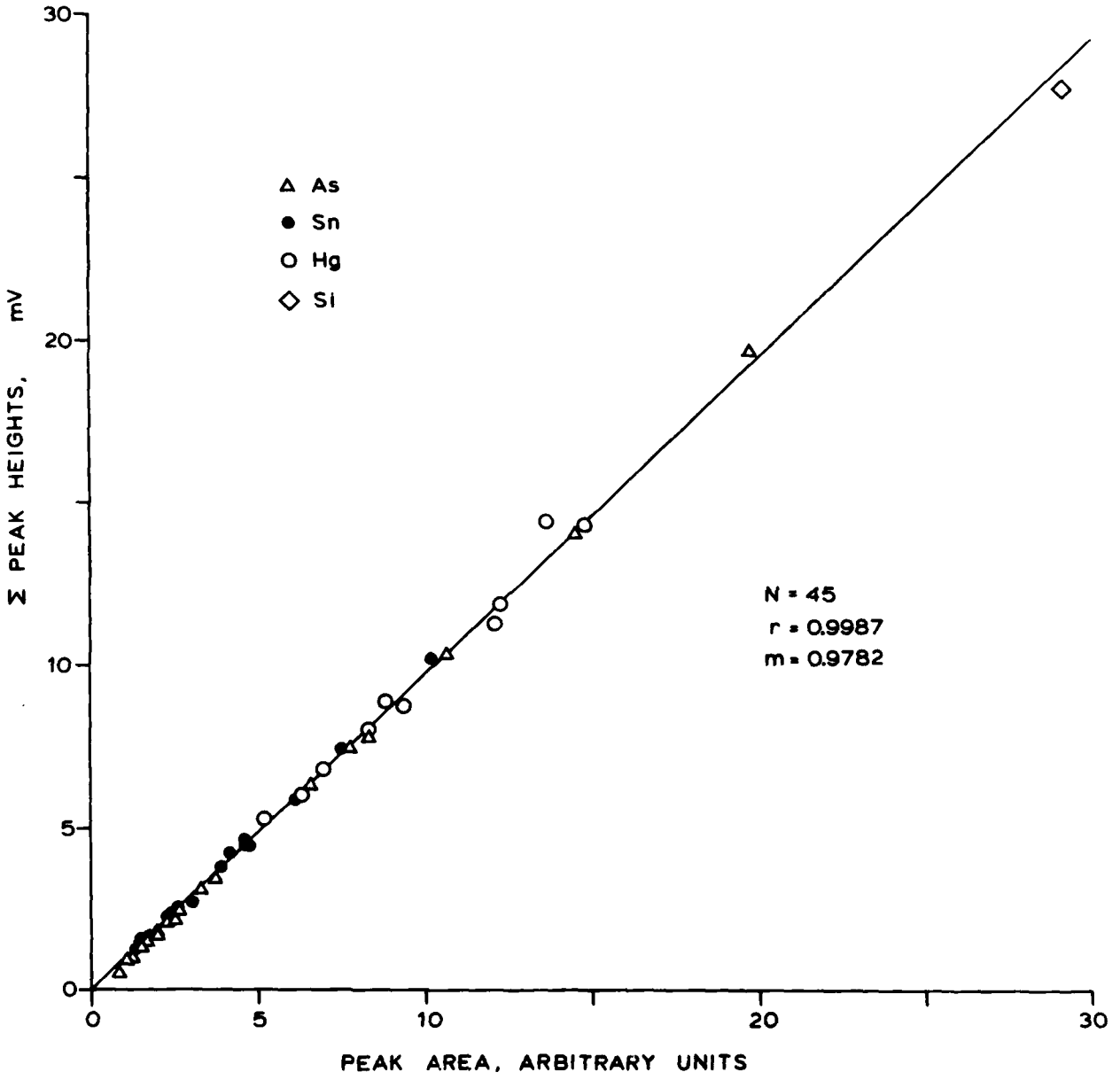
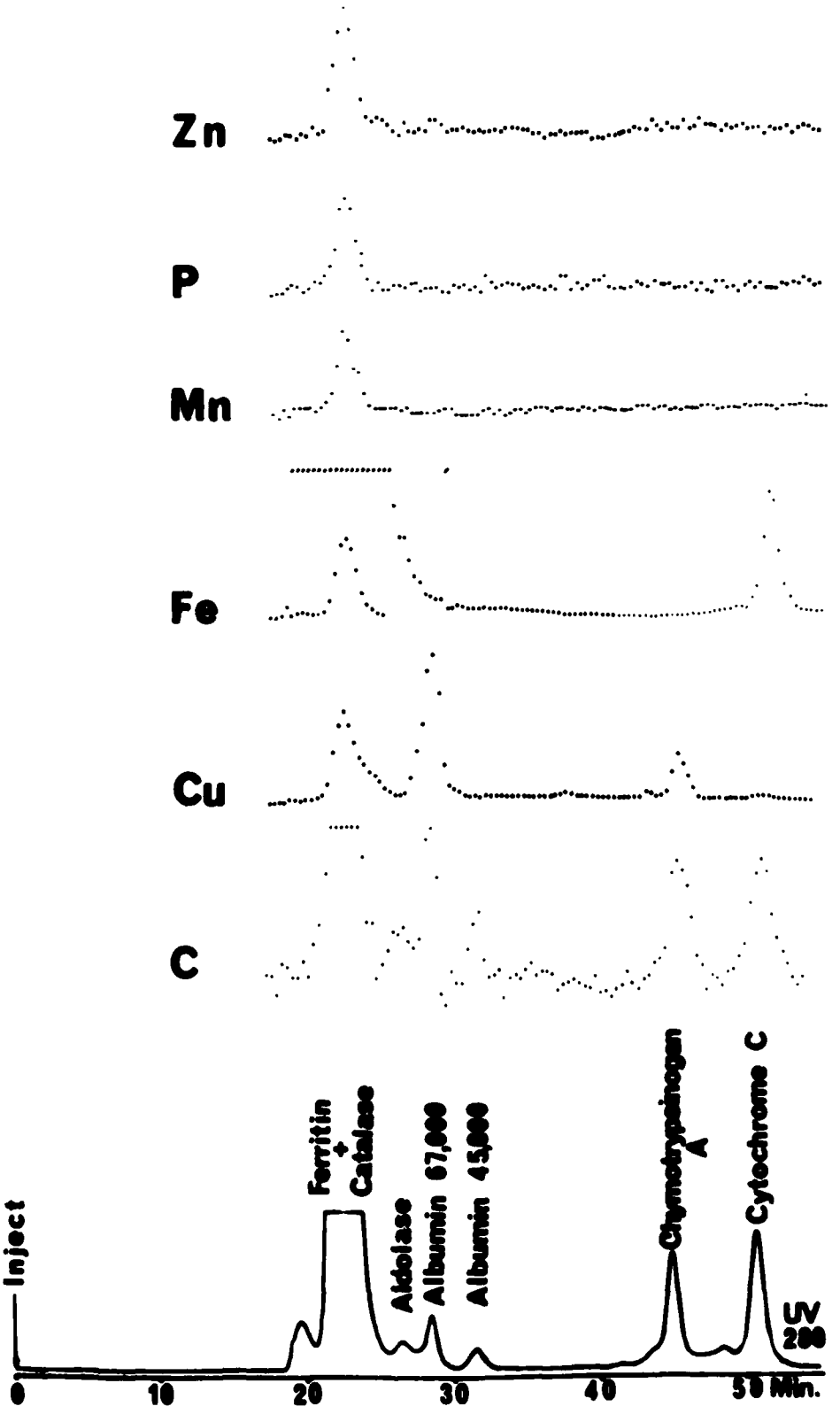

30
20
10
5
0
0
5
10
20
30
Σ PEAK HEIGHTS, mV
PEAK AREA, ARBITRARY UNITS
△ As
● Sn
○ Hg
◇ Si
N = 45
r = 0.9987
m = 0.9782

FIGURE 5


Zn
P
Mn
Fe
Cu
C
Inject
Ferritin
Catalase
Aldolase
Albumin 67,000
Albumin 45,000
Chymotrypsinogen A
Cytochrome C
UV 280
0
10
20
30
40
50 Mn.